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Mitochondrial genome insights into the phylogenetics and biogeographic evolution of snow trout (Cyprinidae, *Schizothorax*) in the Tien Shan Mountains

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Abstract

Snow trout (*Schizothorax*), endemic to the high-altitude freshwater systems of the Tibetan Plateau and the Tien Shan Mountains region, are key components of these ecosystems. This fish lineage may serve as an appropriate model to understand the evolution of biodiversity in the Tien Shan Mountain water ecosystems. However, research has been hindered by poorly understood phylogenetic relationships, unresolved taxonomic classifications, and ambiguous biogeographical histories, particularly in the underexplored western regions of the Tien Shan Mountains. Here, we analyzed three mitochondrial genomes assembled using the next-generation sequencing data of three snow trout species, *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp., from the western Tien Shan Mountains. These genomes range from 16,584 to 16,592 bp in length and include 13 protein-coding genes (PCGs), 2 rRNAs, 22 tRNAs, and a control region. Our phylogenetic analyses suggest six snow trout from the Tien Shan Mountains region were divided into two distinct clades: clade I comprises *S. fedtschenkoi*, and *Schizothorax* sp., and clade II includes *S. eurystomus*, *S. biddulphi*, *S. pseudoaksaiensis*, and *S. argentatus*. We classified *Schizothorax* sp. as a valid independent species based on comprehensive phylogenetic trees and DNA barcode genetic distance. The dramatic uplift of the Tien Shan Mountains during the Late Miocene to the Pliocene, followed by periods of isolation on its eastern and western flanks, has driven extensive speciation, contributing to the rich diversity observed today. Notably, *S. eurystomus* spread westward along the Pamir-Tien Shan corridor, shaping the region's current biogeographical distribution of snow trout species. Our findings not only clarify the evolutionary histories of snow trout in the Tien Shan Mountains but also advance our understanding of the mechanisms shaping the rich biodiversity.

Key Words

DNA barcoding, mitochondrial genome, morphology, phylogenetic relationship, phylogeography, Schizothoracinae

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Introduction

Snow trout of the genus *Schizothorax* are essential faunal elements in the mid-upper reaches of drainages of the Tibetan Plateau (TP), as well as the Tien Shan region to the north of the TP (Wu and Wu 1992; Chen and Cao 2000). Belonging to the primitive group of Schizothoracinae, snow trout are distributed across the cold, high-altitude freshwater ecosystems of both the Tibet Plateau and the Tien Shan Mountain region. The distribution of snow trout across these regions highlights their unique adaptations to extreme environmental conditions. As a basal clade, snow trout species have been profoundly influenced by vicariant speciation, driven by these regions' tectonic uplifts and/or paleoclimate shifts (He and Chen 2006; Chen et al. 2016; Regmi et al. 2023; Gu et al. 2024; Li et al. 2024). This makes them ideal models for investigating the historical biogeography and evolutionary biology patterns within high-altitude ecosystems. However, performing comprehensive and in-depth research on snow trout involves addressing some fundamental issues that still need to be fully resolved. These include taxonomic challenges and phylogenetic relationships among these species, and their biogeography, especially in the less-studied western regions of the Tien Shan Mountain.

Previous studies suggest that the 'Tarim-Central Asia' snow trout represents the most primitive taxa within *Shizothorax*, with the western Tien Shan Mountains potentially being the origin area (He and Chen 2006; Regmi et al. 2023). However, several critical issues remain unresolved within this region's species. One of the main problems is species delimitation (He and Chen 2006), which is often ambiguous due to high levels of morphological similarity among snow trout species. Phylogenetic relationships among snow trout species in the Tien Shan Mountain region remain unclear, primarily due to the lack of comprehensive studies, despite the significant evolutionary importance of these species in this region. Furthermore, the biogeographical evolutionary history of snow trout in the Tien Shan region is still poorly understood. It is still unclear how these species evolved, driven by tectonic activity and climatic fluctuations. A broader comparative analysis involving species across different river drainages in the Tien Shan region could shed light on the taxonomic issues, unresolved phylogenetic relationships, and unclear biogeographical evolutionary histories. These challenges hinder a comprehensive understanding of *Schizothorax* evolution and, by extension, the evolutionary dynamics of the entire Schizothoracinae subfamily.

In this study, we assembled the complete mitochondrial genomes for three snow trout species of *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp. from three river drainages in the western Tien Shan Mountains using next-generation sequencing data. We analyzed the mitogenomic structure, nucleotide composition, codon usage, and tRNA structure. Furthermore, the phylogenetic analyses based on 13 protein-coding genes and the intra- and interspecific genetic distances derived from the *COI* barcode sequences were performed to preferably discuss the phylogenetic relationship among snow trout species distributed in the western Tien Shan Mountains. Finally, we provide the history of the biogeographical evolution of snow trout in the Tien Shan Mountains. Taken together, our results provide a potential detailed evolutionary history of snow trout in the Tien Shan Mountain and offer new insights into understanding the mechanisms driving biodiversity formation in these hotspots.

Materials and methods

Sampling, sequencing, and data collecting

In 2023, samples of three snow trout species—*S. eurystomus* (n = 23), *S. fedtschenkoi* (n = 12), and *Schizothorax* sp. (n = 3)—were collected from the Chirchik River (41°37.6553'N, 69°56.6954'E), Sentobsoy stream (40°36.5486'N, 66°40.4471'E), and Qoratog River, a left tributary of the Surkhandarya River, (38°21.5284'N, 68°4.1839'E) (Fig. 1a), respectively. Samples were collected using trammel and gill nets under standardized field protocols. Sampling and storing procedures adhered to the standard fisheries methods outlined by Kottelat and Freyhof (2007) and Jenkins et al. (2014). All the samples were identified based on morphological characteristics (Berg 1949; Turdakov 1963; Mitrofanov et al. 1988) and stored in 96% ethanol at -20 °C. Whole genome resequencing was performed on one individual from each of the three snow trout species sampled in this study. The Biomarker Technologies Corporation performed genomic DNA extraction, DNA library construction, and sequencing. Briefly, genomic DNA was extracted from ethanol-preserved fin clips, DNA library with an insert size of 300–500 bp was constructed for each species and sequenced on the Illumina NovaSeq platform with a 150 bp paired-end strategy. A total of 2 Gb Illumina paired-end short sequencing data were generated and used for mitochondrial genome assembly.

Mitogenome assembly, annotation, and sequence analysis

The mitogenomes of three snow trout species were assembled using GetOrganelle v1.7.7.0 (Jin et al. 2020), applying 2 Gb of Illumina paired-end sequencing data. Multiple k-mer sizes (21, 35, 45, 65, 85, 105, 121, 135, and 145) were used to optimize assembly accuracy, with a minimum coverage threshold of 10× to ensure high-quality mitochondrial genome reconstruction. The resulting assemblies exhibited average base coverage of 195.9×, 126.1×, and 111.4× for *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp., respectively. Consequently, the assembly was annotated using MitoAnnotator v3.95 (Zhu et al. 2023). The annotated tRNA genes were confirmed, and the secondary

structure was predicted with the tRNAscan-SE search server v2.0 (Chan et al. 2021). The software MEGA11 (Tamura et al. 2021) was used to analyze the nucleotide composition and relative synonymous codon usage (RSCU). To understand the base compositional bias of the mitochondrial genome, AT skew and GC skew were estimated using the following formulae: AT skew = (A-T)/(A+T) and GC skew = (G-C)/(G+C) (Perna and Kocher 1995).

Phylogenetic analysis

To clarify the phylogenetic positions of our three snow trout species within the genus *Schizothorax*, we downloaded the complete mitogenomes of thirty snow trout species and six outgroup species of *Percocypris pingi*, *Gymnodiptychus dybowskii*, *Onychostoma macrolepis*, *Luciobarbus brachycephalus*, *Hypsibarbus vernayi*, and *Pethia ticto* from NCBI (Table 1). Thirteen PCG sequences from thirty-nine individuals representing thirty-seven species were aligned using MUSCLE v3.8.31 (Edgar 2004) with default parameters. Phylogenetic relationships were inferred using the methods of maximum likelihood (ML) and Bayesian inference (BI). The ML phylogenetic tree was constructed with IQ-TREE v2.2.6 (Minh et al. 2020) with the following parameters: "-m MFP+MERGE -B 1000". Using these parameters, the best-fit substitution model models (including FreeRate heterogeneity models) and partition schemes were inferred via the built-in ModelFinder (Kalyaanamoorthy et al. 2017). "MFP" (ModelFinderPlus) allows for extended model selection followed by tree inference, while "-B 1000" ensures that 1000 bootstrap searches will be performed to infer the consensus trees.

Table 1. Complete mitogenome sequences involved in this study.

No.	Species	Accession No.	Subfamily	Reference
1	*Schizothorax labiatus*	KT833091	Schizothoracinae	–
2	*Schizothorax progastus*	KF739399	Schizothoracinae	Goel et al. (2014)
3	*Schizothorax nepalensis*	AP011207	Schizothoracinae	–
4	*Schizothorax niger*	KF600712	Schizothoracinae	Goel et al. (2013)
5	*Schizothorax plagiostomus*	AP011413	Schizothoracinae	–
6	*Schizothorax esocinus*	AP011412	Schizothoracinae	–
7	*Schizothorax richardsonii*	AP011208	Schizothoracinae	–
8	*Schizothorax gongshanensis*	KT946652	Schizothoracinae	Li et al. (2016)
9	*Schizothorax yunnanensis paoshanensis*	KP892531	Schizothoracinae	Li et al. (2016)
10	*Schizothorax nukiangensis*	KT223584	Schizothoracinae	Li et al. (2016)
11	*Schizothorax lantsangensis*	KP143725	Schizothoracinae	Li et al. (2016)
12	*Schizothorax lissolabiata*	KT833094	Schizothoracinae	–
13	*Schizothorax grahami*	KU234535	Schizothoracinae	Zheng et al. (2016)
14	*Schizothorax davidi*	KM879227	Schizothoracinae	Wang et al. (2015)
15	*Schizothorax prenanti*	KT833105	Schizothoracinae	–
16	*Schizothorax dolichonema*	KJ184546	Schizothoracinae	Yue et al. (2014)
17	*Schizothorax taliensis*	MH094667	Schizothoracinae	Rui et al. (2018)
18	*Schizothorax kozlovi*	KJ755668	Schizothoracinae	–
19	*Schizothorax chongi*	KJ718889	Schizothoracinae	Que et al. (2014)
20	*Schizothorax oconnori*	KC513575	Schizothoracinae	Chen et al. (2013a)
21	*Schizothorax wangchiachii*	KC292197	Schizothoracinae	Chen et al. (2013c)
22	*Schizothorax curvilabiatus*	MF804977	Schizothoracinae	Wang et al. (2017)
23	*Schizothorax waltoni*	KC513574	Schizothoracinae	Chen et al. (2013b)
24	*Schizothorax macropogon*	KC020113	Schizothoracinae	Zhu et al. (2013)
25	*Schizothorax integrilabiatus*	MG280782	Schizothoracinae	Zhang et al. (2018)
26	*Schizothorax eurystomus*	PP375290	Schizothoracinae	This study
27	*Schizothorax fedtschenkoi*	PP375291	Schizothoracinae	This study
28	*Schizothorax* sp.	PP375292	Schizothoracinae	This study
29	*Schizothorax eurystomus*	KY436758	Schizothoracinae	–
30	*Schizothorax pseudoaksaiensis*	KM079630	Schizothoracinae	Bao et al. (2016)
31	*Schizothorax biddulphi*	OR812523	Schizothoracinae	–
32	*Schizothorax argentatus*	SRR26304028	Schizothoracinae	Huang et al. (2024)
	Schizothorax argentatus	NC_061395	Schizothoracinae	Wang et al. (2022)
33	*Percocypris pingi*	NC_018601	Schizothoracinae	Li et al. (2013)
34	*Gymnodiptychus dybowskii*	KT588613	Schizothoracinae	Yang et al. (2016)
35	*Onychostoma macrolepis*	MT024680	Acrossocheilinae	Chen and Tang (2020)
36	*Luciobarbus brachycephalus*	NC_056152	Barbinae	Jiang et al. (2019)
37	*Hypsibarbus vernayi*	NC_031621	Cyprininae	–
38	*Pethia ticto*	NC_008658	Barbinae	Saitoh et al. (2006)

The BI analysis was performed in MrBayes v3.2.7a (Ronquist et al. 2012), with the data partitioned by the gene. Before BI analysis, the best-fit model (GTR+I+G) was selected by Akaike Information Criterion (AICc) with jModeltest v2.1.10 (Darriba et al. 2012). Then, BI analysis was carried out using four simultaneous Markov Chain Monte Carlo chains for 1,000,000 generations and sampled every 100 generations, using a burn-in of 25% of the generations. The average standard deviation of split frequencies was < 0.01, showing that the sampling of the posterior distribution was enough. Phylogenetic trees generated from both ML and BI methods were visualized in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Divergence time estimation

The divergence times of *Schizothorax* fishes were estimated using MCMCTree within the PAML v4.10 package (Yang 2007), employing the 13 mitochondrial protein-coding genes and ML tree topology as the input files. We applied the HKY85 substitution model with the overall substitution rate (rgene_gamma) and rate-drift parameter (sigma2_gamma), which were set as G (1, 2) and G (1, 10), respectively. The MCMC run was first executed for 20,000 iterations as burn-in, followed by an additional 100,000 generations with a sample frequency of 10. We used four calibration points: > 0.09 and < 0.20 million years ago (Mya) for the ancestor of *S. davidi* and *S. grahami*; > 2.30 and < 3.23 Mya for the ancestor of *S. yunnanensis paoshanensis* and *S. gongshanensis*; > 0.07 and < 0.17 Mya for the ancestor of *S. wangchiachii* and *S. oconnori*; and > 8.13 and < 11.43 Mya for the common ancestor of *Percocypris pingi* alongside all *Schizothorax* species. These calibration points were derived from the study by Li and Guo (2020). We checked convergence by running all analyses twice and using Tracer v1.7.2 (Rambaut et al. 2018) to monitor effective sample sizes (ESS). ESS values for all parameters over 200 indicated sufficient sampling and good convergence.

Genetic distance calculation

The partial fragments of 652 bp from the 5' end of the mitochondrial *COI* gene were retrieved from the mitochondrial genomes of three newly sequenced species, alongside four previously published mitochondrial genomes of *Schizothorax eurystomus*, *S. fedtschenkoi*, *Schizothorax* sp. (Sheraliev and Peng 2021), *Schizothorax argentatus* (Wang et al. 2022) and *S. pseudoaksaiensis* (Bao et al. 2016). These COI barcode sequences were then aligned, and the pairwise genetic distances between or within species were calculated using the Kimura 2-Parameter (K2P) model (Kimura 1980) implemented in MEGA11 software.

Results and discussion

Mitochondrial organization and nucleotide composition

The complete mitochondrial genomes of *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp. were successfully assembled, revealing a consistent structure across these species. Each genome is a full DNA molecule, with lengths of 16,588 bp, 16,584 bp, and 16,592 bp (Fig. 1b–d), respectively. The genomes contain 37 genes: 13 PCGs, 22 tRNAs, and 2 rRNAs, and a non-coding control region (CR), following the typical vertebrate mitochondrial organization (Fig. 1b–d and Suppl. material 1: table S1). The nucleotide composition of the mitochondrial genome is nearly identical across species, with A (29.4–29.8%), T (25.3–25.6%), G (17.9–18.1%), and C (26.8–27.1%) (Suppl. material 1: table S2). The lengths of intergenic spacers varied from 1 to 34 bp, with notable long intergenic spacers between $tRNA^{Asn}$ and $tRNA^{Cys}$ (34 bp) and $tRNA^{Asp}$ and *COI* (13 bp) (Suppl. material 1: table S1). The thirteen PCGs across the three species are highly conserved. The A+T content for the PCGs is similar, ranging from 54.2% to 54.6% (Suppl. material 1: table S2). Both the AT skew values and GC skew values for the PCGs of three species are nearly identical, with AT skew values of -0.007, -0.007, and 0, and GC skew values of -0.220, -0.218, and -0.211 for *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp., respectively (Suppl. material 1: fig. S1, table S2). The start codon of most PCGs was ATG, but *COI* started with GTG (Suppl. material 1: fig. S2). The standard stop codon (TAA) was used by *ND1*, *COI*, *ND4L*, *ND5*, and *ND6*, and two incomplete codons (T and TA) were used by *ND2*, *COII*, *ATPase6*, *COIII*, *ND3*, *ND4*, and *Cyt b* (Suppl. material 1: table S1). The 13 protein-coding genes expressed 5,242, 5,018, and 5,111 amino acid triplets, respectively, excluding the stop codon (Suppl. material 1: table S3). These mitochondrial genomes also have one *12S rRNA* and one *16S rRNA* gene, located between $tRNA^{Phe}$ and $tRNA^{Val}$, and between $tRNA^{Val}$ and $tRNA^{Leu(UUR}$ (Fig. 1b–d and Suppl. material 1: table S1), respectively. The size of the *12S rRNA* were 956 bp, 956 bp, and 955 bp, and the size of the *16S rRNA* were 1677 bp, 1678 bp, and 1679 bp in *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp., respectively (Suppl. material 1: table S1). In *Schizothorax* sp., the A+T content of the rRNA genes was 54%, higher than in *S. eurystomus* (46.3%) and *S. fedtschenkoi* (46.2%) (Suppl. material 1: table S2). All three species showed a positive AT skew (0.270, 0.260, and 0.263, respectively) of the rRNA and a negative GC skew (Suppl. material 1: fig. S1, table S2), indicating higher A and C content than T and G content in the *rRNAs* (Suppl. material 1: table S2). The tRNA genes ranged in length from 67 ($tRNA^{Cys}$) to 76 bp ($tRNA^{Lys}$) (Suppl. material 1: table S1). Of these, 14 tRNA genes were located on the heavy (+) strand, and 8 tRNA genes were located on the light (–) strand (Fig. 1b–d and

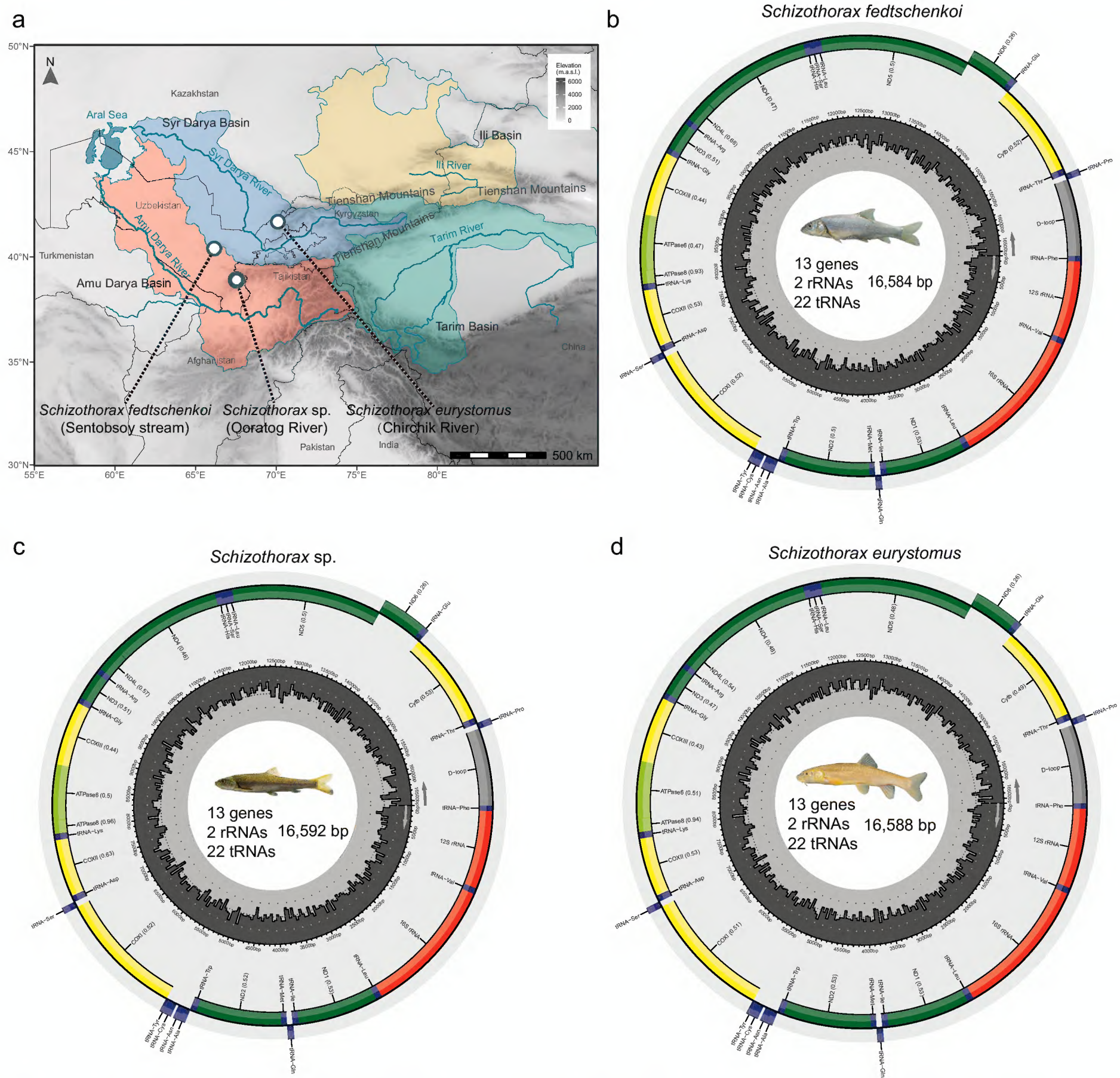


Figure 1. The sampling sites and mitochondrial genome maps of the three *Schizothorax* species. **a.** The sampling sites of three snow trout species. Amu Darya Basin, Syr Darya Basin, Tarim Basin, and Ili Basin, were shown in different colors; **b–d.** The mitochondrial genome map of *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp, respectively.

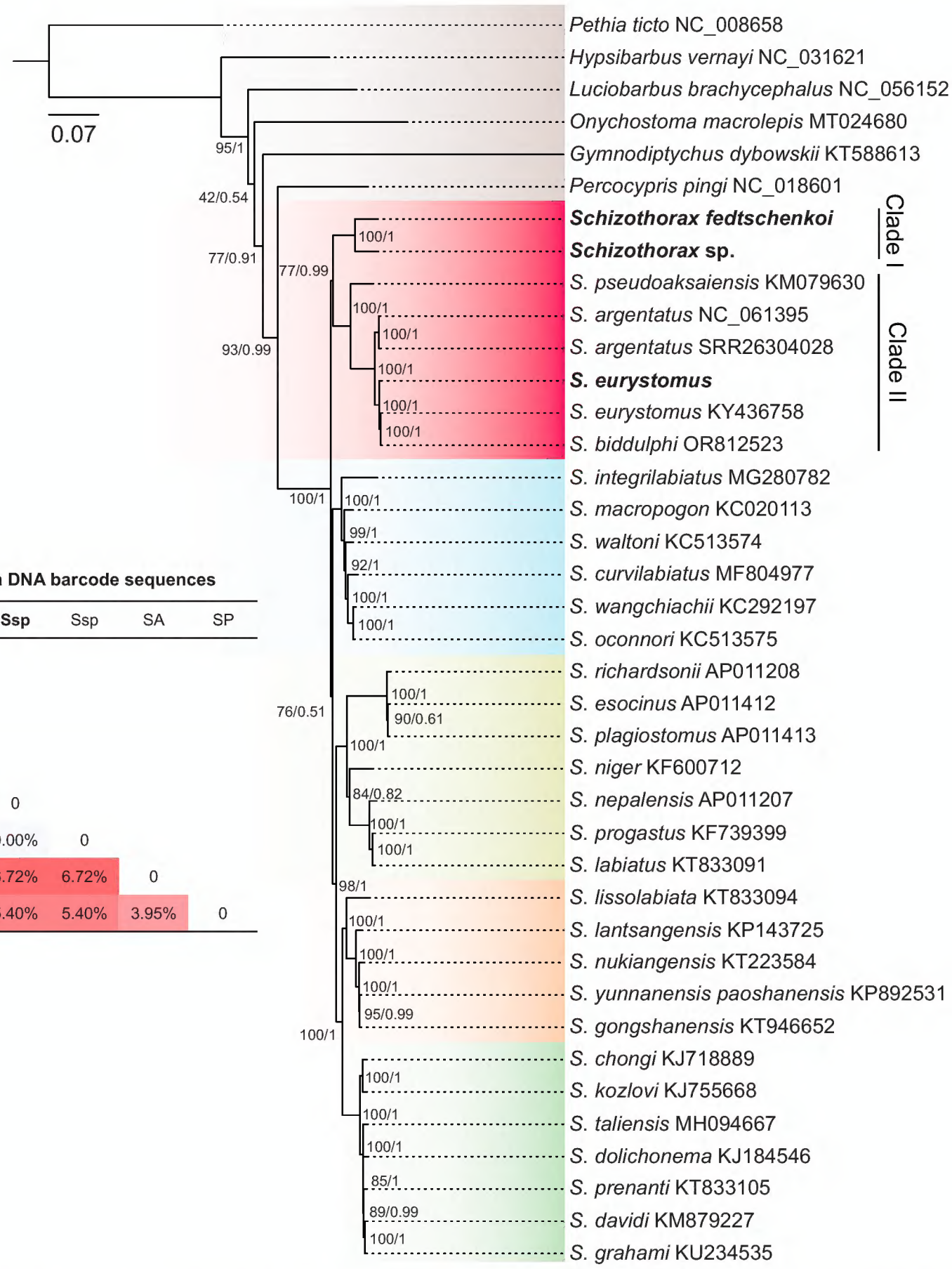
Suppl. material 1: table S1). All the tRNAs displayed the clover-leaf secondary structure (Suppl. material 1: fig. S3), excluding the $tRNA^{Ser(AGY)}$, which cannot form a stable secondary structure because of the lack of the DHU (dihydrouridine) arm (Suppl. material 1: fig. S3). This loss was reported in many fishes, such as *Alcichthys elongatus*, *Linichthys laticeps*, and *Sebasticus marmoratus* (Jia et al. 2020; Patil et al. 2023; Zhang et al. 2023).

Phylogenetic relationships and COI barcode sequence-based genetic distance

We used 13 mitochondrial PCGs of 31 *Schizothorax* species and six outgroup species to build the BI and ML trees to determine phylogenetic relationships for the three snow trout. The BI and ML trees were resolved into five distinct clusters (Fig. 2a), supporting the results of previous studies (He and Chen 2006; Yang et al. 2012) and confirming the consistency of the observed phylogenetic patterns. The phylogenetic tree showed snow trout from the Tien Shan Mountains group into two distinct clades, I and II. Clade I comprises a pair of sister species, *S. fedtschenkoi* and *Schizothorax* sp. (Fig. 2a), primarily distributed in the western Tien Shan Mountains, specifically in the right tributaries of the Amu Darya River. Clade II includes species from other regions of the Tien Shan Mountains (Fig. 2a).

Interestingly, our findings reveal that two individuals of *S. eurystomus* do not form a monophyletic group. One individual of *S. eurystomus* from the Tarim

a



b

Genetic distances calculated based on DNA barcode sequences

	SE	SE	**SF**	SF	**Ssp**	Ssp	SA	SP
SE	0							
SE	0.15%	0						
SF	6.72%	6.55%	0					
SF	6.72%	6.55%	0.29%	0				
Ssp	6.55%	6.39%	3.18%	3.18%	0			
Ssp	6.55%	6.39%	3.18%	3.18%	0.00%	0		
SA	1.04%	0.89%	6.22%	6.22%	6.72%	6.72%	0	
SP	3.80%	3.64%	5.24%	5.24%	5.40%	5.40%	3.95%	0

Figure 2. Maximum likelihood and Bayesian inference phylogenetic tree of 33 *Schizothorax* fishes based on 13 protein-coding genes. **a.** Maximum likelihood phylogenetic tree. The numbers at the nodes indicate the bootstrap value for the maximum likelihood tree and the posterior probability for the Bayesian inference tree, respectively. Tip labels in bold indicate those for which whole genome sequencing was conducted in this study; **b.** Genetic distances calculated based on DNA barcode sequences. The bolded species names indicate the sequenced species or individual in this study, while the regular species names indicate the species or individual for which NCBI data was obtained. **SE**, *S. eurystomus*; **SF**, *S. fedtschenkoi*; **Ssp**, *Schizothorax* sp.; SE, *S. eurystomus* KY436758; SF, *S. fedtschenkoi* (not displayed in the tree); Ssp, *Schizothorax* sp. (not displayed in the tree); SP, *S. pseudoaksaiensis* KM079630; SA, *S. argentatus* SRR26304028.

Basin (KY436758) and the individual of *S. biddulphi* (OR812523) from the same basin are sister taxa, jointly forming a clade with one individual of *S. eurystomus* sampled from the western Tien Shan Mountains. Although incomplete lineage sorting and hybridization might both account for this observation, we propose that interspecific hybridization between *S. eurystomus* and *S. biddulphi* is likely the main driver, given that reproductive isolation is not absolute among congeneric fishes (Campton and Utter 1985; Müller et al. 2010; Ma et al. 2018) and the two species share not only overlapping habitats but also breeding seasons (Guo et al. 2012). Further supporting our hypothesis, a study based on the *COI* gene revealed significant gene flow among four snow trout species (*S. eurystomus*, *S. biddulphi*, *S. irregularis*, and *S. barbatus*) in the major tributaries of the Tarim River (Haysa et al. 2021). Future research based on population genomics will

provide deeper insights into the hybridization history of these fish species, enhancing our understanding of their evolutionary dynamics.

Notably, the phylogenetic analyses distinctly identified *Schizothorax* sp. as an independent species within the evolutionary tree, underscoring its unique taxonomic status. The K2P distance between *Schizothorax* sp. and other valid species ranges from 3.18% to 6.72%, while the distance between our sequenced *Schizothorax* sp. individual and that from previous study (Sheraliev and Peng 2021) is 0 (Fig. 2b). These results confirm its status as a valid independent species, meeting the established criterion of a genetic distance greater than a 2% interspecific distance and less than a 1% intraspecific distance, as outlined by Hubert and Hanner (2015). However, since the K2P genetic distance calculations did not account for variability, there may be issues with insufficient statistical robustness.

Future research should employ population genomics and large-scale sampling to deepen our understanding of the hybridization history and evolutionary dynamics of these snow trout species in the Tien Shan Mountains. Nuclear genome-level studies are critical to overcome the limitations and potential inaccuracies of phylogenetic inferences based solely on mitochondrial DNA, particularly in polyploid species with complex evolutionary histories. Moreover, population-level investigations using genomic data are essential for uncovering finer-scale hybridization patterns and offering more profound insights into their speciation processes and adaptive evolution.

Divergence time estimation and biogeography history inference

Notably, the phylogeny based on the 13 protein-coding genes revealed that snow trout species from the Tien Shan Mountains formed a well-supported monophyletic clade (bootstrap value of 100 for ML tree and posterior probability of 1 for BI tree) (Fig. 2a). This clade originated around 7.12 Mya with 95% confidence interval (CI) of 8.46–5.70 Mya (Fig. 3). This clade subsequently diverged into two distinct clades (I and II) (Fig. 3) around 6.41 Mya (95% CI: 8.01–4.75 Mya). Clade I, which comprises *Schizothorax* sp. and *S. fedtschenkoi*, both endemic to the right tributaries of the Amu Darya River, diverged at 3.01 Mya (95% CI: 5.42–1.07 Mya) (Fig. 3). In clade II, *S. pseudoaksaiensis* from the Ili River, *S. argentatus* from the northern Tien Shan Mountains, and *S. eurystomus* from the Tarim Basin independently emerged at 3.91 Mya (95% CI: 5.97–1.78 Mya) and 1.31 Mya (95% CI: 2.29–0.45 Mya) (Fig. 3), respectively. Then, *S. eurystomus* diverged from *S. biddulphi* approximately 0.41 Mya (95% CI: 0.91–0.09 Mya) and then spread from the eastern Tien Shan Mountains to the western Tien Shan Mountains. The timing of these cladogenetic events coincides with the period of significant uplift of the Tien Shan Mountains that occurred 7.00–2.58 Mya (Sun et al. 2004). We infer that allopatric divergence of snow trout was likely driven by vicariance associated with the rapid orogeny of the Tien Shan Mountains from the Late Miocene to the Pliocene. Similar allopatric divergence patterns have been observed in other species, such as sand snake *Psammophis turpanensis* (Chen et al. 2021).

The uplift of the Tien Shan Mountain and its adjacent area had driven the diversification of these snow trout (Fig. 4). The extensive uplift of the Tien Mountain during the Late Miocene to the Pliocene separated the Amu Darya drainage and the Syr Darya drainage from the Tarim Basin, which divided the ancestor of these snow trout species into two groups, A1 and A2 (Figs 3, 4a, b). A1 is the ancestor of *S. fedtschenkoi* and *Schizothorax* sp., and A2 is the ancestor of *S. pseudoaksaiensis*, *S. argentatus*, *S. eurystomus*, and *S. biddulphi*. The orogeny of the Pamir-Alai mountains, western part of the Tien Shan Mountains, induced geographical isolation that led to the speciation within A1, resulting in two distinct species, *S. fedtschenkoi* and *Schizothorax* sp. (Fig. 4c). Concurrently, the uplift of the Tien Shan Mountains, led to the formation of the Ili River valley, sequentially triggered speciation processed within A2, leading to the emergence of *S. pseudoaksaiensis* (Fig. 4c), *S. argentatus*, and *S. eurystomus* (Fig. 4d). After that, *S. eurystomus* dispersed into the Syr Darya Basin along the Pamir-Tien Shan corridor, establishing the current biogeographic distribution pattern on both sides of the Tien Shan Mountains. Subsequent uplifts of the Tien Shan and the resulting changes in river systems facilitated the divergence of *S. biddulphi* from *S. eurystomus*, which is distributed in the Tarim Basin (Fig. 4e).

The uplift of the Tien Shan Mountains has significantly influenced several species' evolutionary trajectories and biogeographic patterns across this region. Similar to the observed allopatric divergences and origins in snow trout in this study, the uplift has influenced other taxa in comparable ways. For example, population differentiation in *Meriones meridianus* was driven by the uplift of the Tien Shan Mountains during the Middle Pleistocene (Wang et al. 2013), while amphipods, such as *Gammarus lacustris*, originated in the Tien Shan region and subsequently dispersed through alpine lakes to high-latitude and high-altitude areas across the Northern Hemisphere (Hou et al. 2022). Our study demonstrates that the uplift of the Tien Shan Mountains has played an essential role in shaping regional biodiversity through allopatric speciation, impacting both in the range's western and eastern sections.

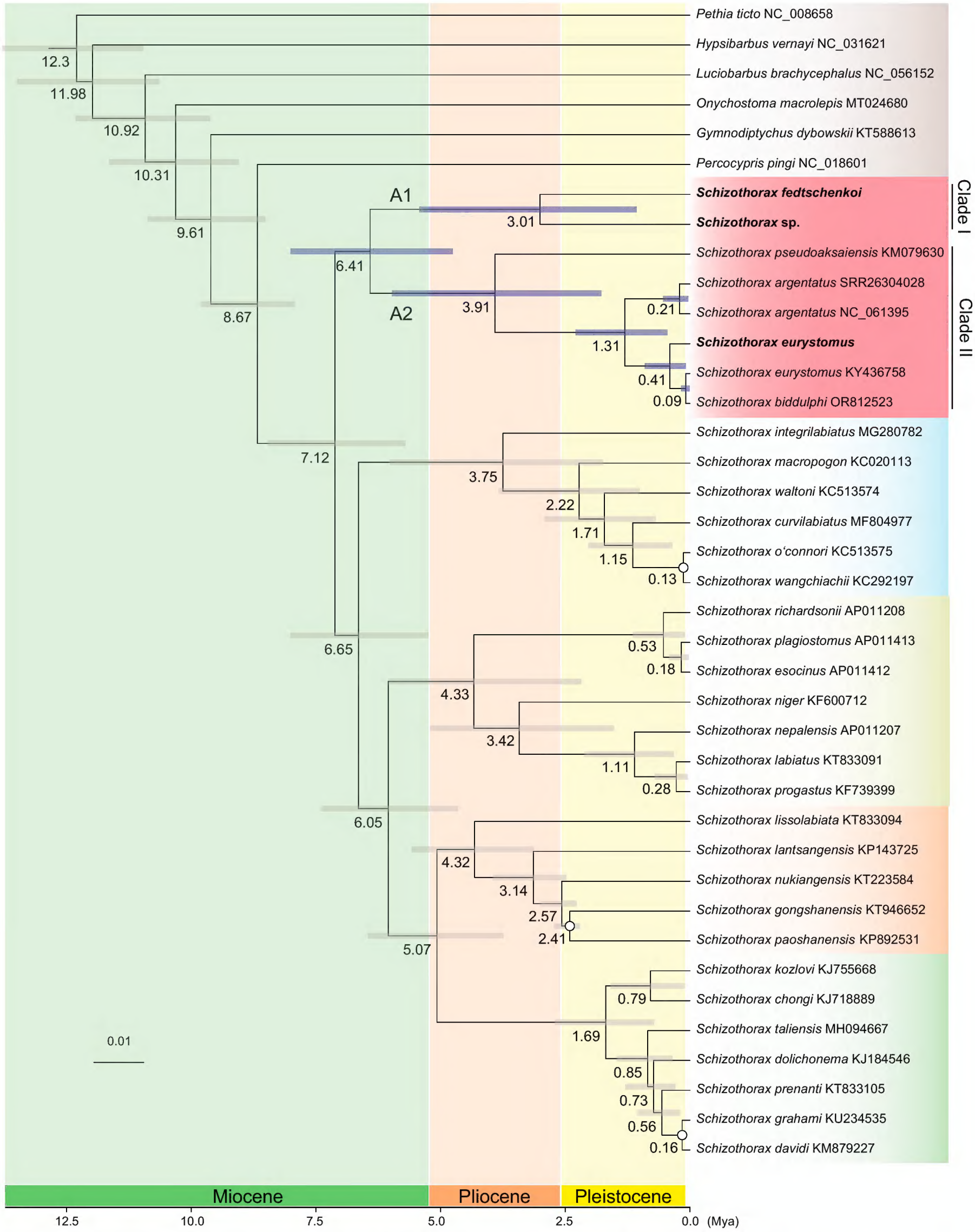


Figure 3. Time-calibrated phylogenetic tree of 33 *Schizothorax* species based on ML topology from 13 mitochondrial protein-coding genes. White circles with black borders represent calibrated nodes. The bolded species names indicate the sequenced species or individual in this study, while the regular species names indicate the species or individual for which NCBI data was obtained.

Conclusion

This study sequenced and assembled the mitochondrial genomes of three snow trout species, *S. eurystomus*, *S. fedtschenkoi*, and *Schizothorax* sp., from the western Tien Shan Mountains. These genomes range from 16,584 to 16,592 bp and include 13 protein-coding genes, two rRNAs, 22 tRNAs with a clover-leaf structure except $tRNA^{Ser(AGY)}$, and a control region. Phylogenetic analyses suggest six snow trout species from the Tien Shan

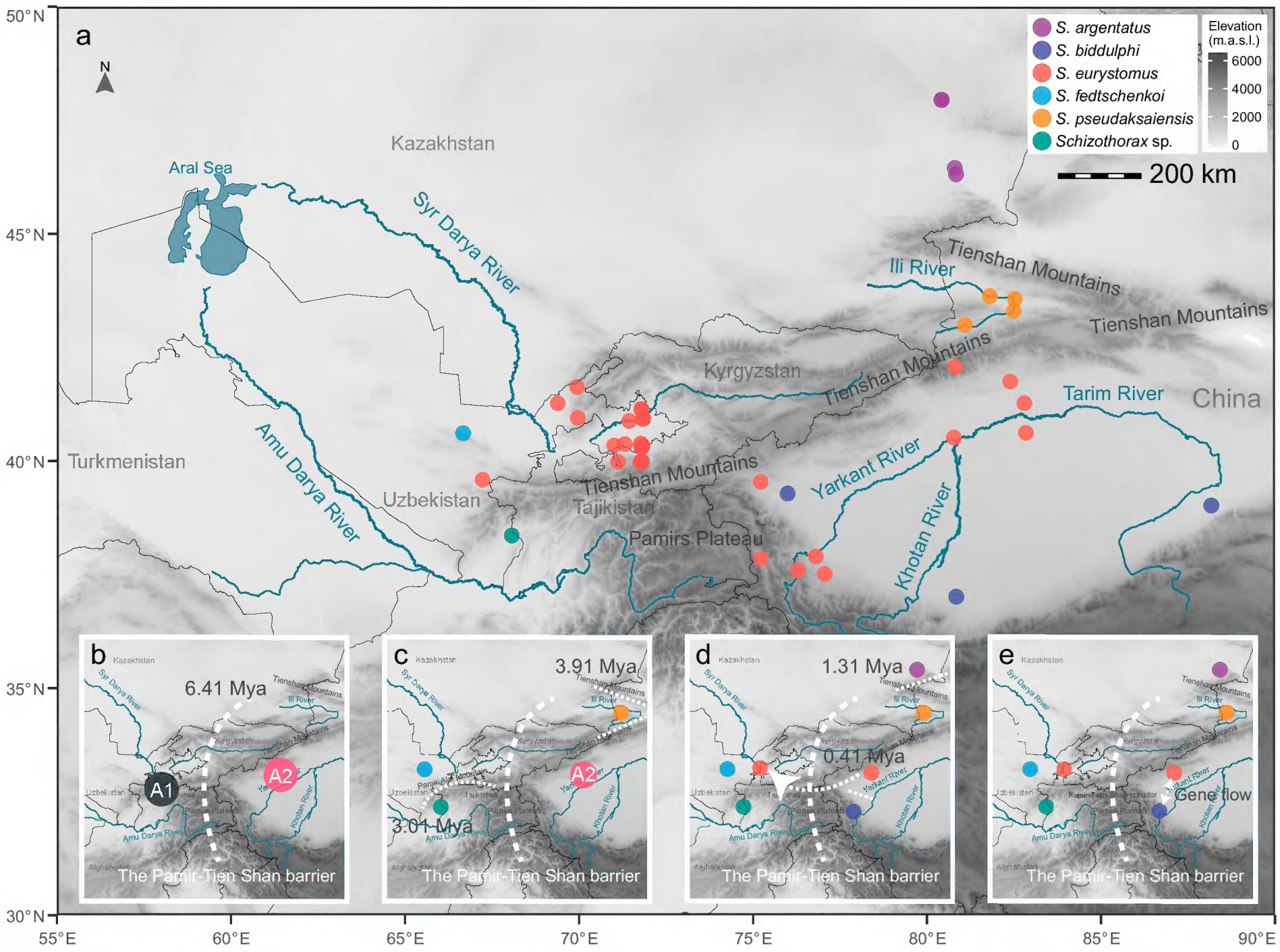


Figure 4. The biogeography history of snow trout in the Tien Shan Mountains. **a.** Current geographic distribution of these species. Occurrence data were compiled from the Global Biodiversity Information Facility (GBIF) and our field sampling sites; **b.** The barrier of the Pamirs-Tien Shan separated the ancestor of snow trout in the Tien Shan Mountains into two groups, A1 and A2, approximately 6.41 Mya. A1 is the ancestor of *S. fedtschenkoi* and *Schizothorax* sp., and A2 is the ancestor of *S. pseudoaksaiensis*, *S. argentatus*, *S. eurystomus*, and *S. biddulphi*. Thick white dashed lines indicate the barrier of the Pamirs-Tien Shan; **c.** The uplift of the northern Tien Shan Mountains around 3.91Mya facilitated the geographical isolation and subsequent speciation of *S. pseudoaksaiensis* from A2 along the Ili River. Additionally, the uplift of the Pamir-Alai mountains around 3.01 Mya prompted allopatric isolation, resulting in A1 diverging into two distinct species, *S. fedtschenkoi* and *Schizothorax* sp. Thin white dashed lines indicate different parts of the Tien Shan Mountains; **d.** Further uplift of the Tien Shan Mountains around 1.31 Mya and 0.41 Mya triggered allopatric speciation within A2, leading to the speciation of *S. argentatus* in the northern Tien Shan and *S. eurystomus* in the southern Tien Shan, respectively. Subsequently, *S. eurystomus* spread westward along the Pamir-Tien Shan corridor, resulting in the current geographic distribution pattern that crosses the Pamir-Tien Shan barrier. The white arrow indicates the dispersal event of *S. eurystomus* from east to west across the Pamir-Tien Shan barrier 0.41 Mya. **e.** The potential interspecific gene flow between *S. biddulphi* and the eastern population of *S. eurystomus*. The thin white dashed line with bidirectional arrows indicates the possible gene flow occurred between the two species.

Mountains are clustered into two distinct clades: clades I and II. Clade I comprises *S. fedtschenkoi* and *Schizothorax* sp., while clade II includes *S. eurystomus*, *S. biddulphi*, *S. pseudoaksaiensis*, and *S. argentatus*. The dramatic uplift of the Pamir-Tien Shan range and subsequent isolation along its flanks have driven extensive allopatric speciation, enriching the region's biodiversity. Finally, *S. eurystomus* spread westward along the Pamir-Tien Shan corridor, shaping the current biogeographical distribution of snow trout in the Tien Shan Mountains. Given the limitation of the mitochondrial DNA, potential hybridization event, and the polyploid nature of snow trout species, future research should leverage large-scale population genomic data to further unravel the evolutionary trajectories of these species from a polyploidy perspective.

Data availability statement

The mitogenome sequence data are openly available in the GenBank of NCBI under accession numbers PP375290–PP375292.

Authors' contribution

B.G. and X.L. conceived this study; A.R., X.L., Y.W., M.W., C.W., and M.Z. performed analyses; X.L., A.R., and B.G. wrote the manuscript; A.R., J.S., B.K., E.K., J.F., and S.N. collect the samples; and all authors read and approved the final manuscript.

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **fig. S1.** The mitochondrial genome maps and the nucleotide skewness of the three *Schizothorax* species. The nucleotide skewness for whole mitogenome, PCGs, tRNAs, rRNAs, and control region of three *Schizothorax* species. **fig. 2.** The relative synonymous codon usage (RSCU) in the mitochondrial protein-coding genes of the three *Schizothorax* species. (a) *S. eurystomus*; (b) *S. fedtschenkoi*, (c) *Schizothorax* sp. Codon families are labeled on the x-axis. The termination codon is not given. **fig. S3.** The inferred secondary structures of 22 tRNA genes in the three *Schizothorax* species. The base structure belongs to *S. eurystomus*, while the blue and yellow dots indicate the difference between *S. fedtschenkoi* and *S. eurystomus*, and between *Schizothorax* sp. and *S. eurystomus*, respectively. **table S1.** Gene/element features of *S. eurystomus* (SE), *S. fedtschenkoi* (SF), and *Schizothorax* sp. (Ssp). **table S2.** Nucleotide composition and skewness of the complete mitochondrial genome, protein-coding genes (PCGs), tRNAs, rRNAs, and control region of three *Schizothorax* species. **table S3.** Codon and relative synonymous codon usage (RSCU) of 13 protein-coding genes (PCGs) in three *Schizothorax* species.

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